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BASIC INVESTIGATION

Experimental study on anaphylaxis of Qingkailing injection and its components on Beagle dogs

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Abstract

OBJECTIVE: To study the anaphylaxis of Qingkailing injection (QI) and its components.

METHODS: Experimental anaphylactoid and allergic reactions were used. Changes in the behaviors of Beagles and serum levels of histamine, immunoglobulin (Ig)E, IgG, IgM, eosinophil cationic protein (ECP), and interleukin (IL)-4, as well as blood pressure, after injecting QI and its components on the forelimb veins of Beagles were observed.

RESULTS: According to comprehensive determination of abnormal behavior scores and changes in serum levels of histamine, IgE, IgG, IgM, ECP, and IL-4, as well as in blood pressure, radix isatidis and hyodeoxycholic acid caused anaphylactoid reactions, and honeysuckle, radix isatidis, hydrolysate, cholic acid and Gardenia jasminoides caused allergic reactions. The anaphylaxis of QI involved anaphylactoid and allergic reactions.

CONCLUSION: QI and its components need to be refined further to improve the safety, efficacy, and quality of its use in clinical settings.

Key words: Anaphylaxis; Qingkailing Injection; Composition; Behavior, animal; Immunoglobulin isotypes; Eosinophil cationic protein; Interleukin-4

INTRODUCTION

Traditional Chinese medicine injections (TCMIs) play an important part in the treatment of diseases because of their high bioavailability as well as their rapid and strong action. They are used extensively. However, with the quick development and wide application of TCMIs, reports on their adverse drug reactions (ADRs) are increasing.[1]

The ADRs of TCMIs account for>70% of all ADRs caused by traditional Chinese medicines (TCMs).[2] Anaphylactoid and allergic reactions are important ADRs. These reactions are characterized by rapid onset, so there may not be sufficient time to save the patient. In recent years, the number of clinical reports focusing on this issue has been increasing.[3]

In the present study, the anaphylaxis of Qingkailing injection (QI) and its components were used to provide the theoretical and experimental basis for their clinical applications.

QI is a pure herbal compound preparation comprising Gardenia jasminoides, radix isatidis, honeysuckle, baicalin, cholic acid, hyodeoxycholic acid, buffalo horn, and clam shell. QI can clear heat, remove toxins, dissolve phlegm, and aid resuscitation. It is used to treat: coma caused by fever; paralysis caused by stroke; acute and chronic hepatitis; infection of the upper respiratory tract; pneumonia; and cerebrovascular disease.[4]

Along with the extensive use of QI for clinical treatment, its ADRs have been reported. The multiplicity, universality, unpredictability, and symptom diversity of its ADR have been reported.

MATERIALS AND METHODS

Drugs

Gardenia jasminoides, radix isatidis, honeysuckle, baicalin, cholic acid, hyodeoxycholic acid, hydrolysate (extract from buffalo horn and clam shell) and QI (batch numbers ZHZ-20110720, BLG-20110720, JYH-20110720, HQG-20110721, CA-20110721, HD-CA-20110721, SHJ-20110721, and QKL-20110720) were employed. The drugs were prepared and provided by Yabao Beizhongda (Beijing) Pharmaceutical Co., Ltd. (Beijing, China).

Reagents

Histamine phosphate (crystallization with water; batch number F20080730; Sinopharm Chemical Reagent Co. Ltd., (Beijing, China) was prepared with saline to 1.44% concentration before use. Egg protein powder (batch number 026K5448; Sigma-Aldrich, St Louis, MO, USA) was prepared with saline to 1% concentration before use. Normal saline (NS; batch number 708203) was from Beijing Double-Crane Pharmaceutical Co. Ltd. (Beijing, China). Enzyme-linked immunosorbent assay (ELISA) kits for histamine, immunoglobulin (Ig)E, IgG, IgM, eosinophil cationic protein (ECP), interleukin (IL)-4, 96T (batch numbers 07011102, 07011101, 06301101, 06271101, 06281103, and 06301103, respectively; Rapid BioLab. Calabasas, CA, USA) were provided by Shang-Bai Biomedical Technology Co. Ltd. (Beijing, China).

Instruments

The main instruments were a computerized microinjection pump (AJ-5803, Shanghai AnJie Electronic Equipment Co. Ltd., Shanghai, China) and an Automatic Microplate Reader (550, BioRad, Foster City, CA, USA). A non-invasive Electronic Sphygmomanometer (BP-98E, Softron, Tokyo, Japan) was also used.

Animals

Male and female Beagle dogs [(14.0±0.5) kg] were provided by the Animal Experimental Center of the Academy of Military Medical Science (Beijing, China); Laboratory animal license, SCXK (Army) 2007-0004). The temperature in the experimental room was 20°C-24°C and relative humidity was 48%-58%.

Grouping

Anaphylactoid reaction: 30 Beagles were randomized equally into 10 groups. The groups were: negative control (NS), positive control (histamine phosphate), baicalin, honeysuckle, radix isatidis, Gardenia jasminoides, hydrolysate, cholic acid, hyodeoxycholic acid, and QI.

Allergic reaction: 30 Beagles were randomized equally into 10 groups. The groups were as stated above except the positive control group (egg protein).

Experiment

Anaphylactoid experiment: Beagles in each group were given the corresponding injection at 5 mL/min in the forelimb vein. The volume of injection was 6.67 mL/kg (equivalent to 10 times the adult dose). Changes in the behavior of dogs of each group were observed continuously before and after injection and scored according to the symptoms shown in Table 1. Venous blood (4 mL) was obtained from each dog and centrifuged at 3000 rpm for 10 min at room temperature to ascertain the level of histamine in plasma. Arterial blood pressure was measured non-invasively in the forelimb before and after injection. Results were evaluated according to abnormal behavior scores and histamine increase.

Allergy experiment: on the first, third, and fifth day of the experiment, Beagles in each group were given the corresponding injection at 5 mL/min in the forelimb vein. The volume of the injection was 6.67 mL/kg (three times the injection for sensitization). On the fourteenth day of the experiment, a stimulating intravenous injection was applied with a twofold dose of injection for sensitization (13.34 mL/kg). Changes in the behavior of dogs in each group were observed continuously before and after injection and scored according to the symptoms shown in Table 1. Four milliliters of venous blood was obtained from each dog before the first injection for sensitization and 10 min after the stimulating injection. This blood was centrifuged at 3000 rpm for 10 min at room temperature to detect the plasma levels of histamine, IgE, IgG, IgM, ECP, and IL-4. The arterial blood pressure was measured non-invasively on the forelimb before and after the first and the fourth injections. The results were evaluated according to the abnormal behavior scores and increase in the plasma levels of histamine, IgE, IgG, IgM, ECP, and IL-4.

Statistical analysis

Data are the mean±standard ($\bar{x}$ ±s). One-way ANOVA was used for between-group comparison. The Student-Newman-Keuls test was used to test for homogeneity; the Tamhane T2 test was used to ascertain heterogeneity. SPSS 16.0 software was used for data anayses. P<0.05 was considered significant.

RESULTS

Effect of the anaphylactoid reaction on Beagle dogs

All of the Beagles died (severe behavioral change) after injection in the histamine phosphate group. The level of histamine increased by >100% and the blood pressure decreased significantly compared with the NS group. The Beagles had obvious behavioral changes, and the level of histamine was increased by >100% in the QI group. The Beagles had behavioral changes in the radix isatidis, Gardenia jasminoides, and hydrolysate groups. The level of histamine also increased by > 100% in the radix isatidis and hyodeoxycholic acid groups.

According to the evaluation criteria of the anaphylac-

Table 1 Rating criteria of abnormal behavior[5]

Typical symptoms		Remarks
−	No symptoms	Characteristics: 1. Serious adverse reactions appear in the first time of injection. 2. Typical and multiple symptoms last for over 3 minutes and get gradually worse, even cause death.
+	Expansion of superficial vessels, such as the mouth, ear, nose, and skin flushing	
++	Uneasiness, head hitting against the wall, unsteady standing, falling repeatedly, vomiting repeatedly, salivation, incontinent urination and defecation	
+++	Blood pressure going down with shock-like symptoms, such as abrupt breathing and heartbeating, dark purple or pale skin, cold limbs, and weak pulse. In case symptoms are progressing, there will be respiratory failure.	
++++	Death	

toid reaction in Table 2, the anaphylactoid reaction was strongly positive or positive in the histamine phosphate and QI groups, suspect positive in the radix isatidis and hyodeoxycholic acid groups, and negative in the other groups (Table 3).

Table 2 Rating criteria of anaphylactoid reaction

Abnormal behavior score	Histamine increase (%)	Outcome
<1	0-30	Negative
1-2	31-50	Suspect
2-3	51-100	Positive
>3	>100	Strong positive

Notes: The rating criteria of IgE, IgG, IgM, ECP, and IL-4 in allergic reactions are the same as those of histamine in Table 1.

Effect of the allergic reaction on Beagle dogs

All the Beagles in the egg protein group had severe behavioral responses; the level of serum histamine and IgE increased by >100% and blood pressure decreased significantly, compared with the NS group. The Beagles had obvious behavioral responses in the QI group: the level of serum histamine increased by >50%. There were also obvious behavioral changes in the honeysuckle, radix isatidis, hydrolysate, and cholic acid groups, and the level of serum histamine increased by >50% in the honeysuckle, radix isatidis, Gardenia jasminoides, hydrolysate, and cholic acid groups.

According to the evaluation criteria of the allergic reaction shown in Table 2, the allergic reaction was strongly positive in the egg protein group, positive in the QI, honeysuckle, radix isatidis, hydrolysate and cholic acid groups, suspect positive in the Gardenia jasminoides group, and negative in the other groups (Table 4).

DISCUSSION

TCMIs cause serious ADRs, including anaphylactoid reactions and type-I hypersensitivity, and the former accounts for about 77% of ADRs.[7]

The anaphylactoid reaction is not an antigen − antibody reaction, and the allergen is not necessarily a complete antigen or hapten. It is evoked at the first injection, which directly stimulates mast cells and basophils to release histamine, so allergic-like symptoms are observed. The anaphylactoid reaction is correlated with mast-cell degranulation and histamine release without the need for mediation by IgE. Histamine release is not caused by the antigen - antibody reaction, but the signs and symptoms appear to be those of the allergic reaction (e.g., skin flushing, headache, edema, hypotension, shock). The main agent causing the anaphylactoid reaction is histamine, the clinical symptoms of which are related to the histamine concentration in blood: no symptoms if histamine ≤1 ng/mL, only skin response if 1-2 ng/mL, systemic reaction if 3 ng/mL, and severe reaction if >100 ng/mL (mainly manifested as cardiovascular and respiratory symptoms (allergic shock)).[8]

Type-I hypersensitivity is caused by IgE. Antigen-specific IgE is generated upon first contact with antigen, and the body is sensitized. If the same allergen enters the body, it can be combined with IgE on the surface of the target cell and then type-I hypersensitivity results.[9] Hypersensitivity occurs within minutes after exposure to allergen, and lasts for 30-60 min (immediate hypersensitivity) or happens after stimulation within 2-8 h, and lasts for 1-2 days or longer (delayed hypersensitivity). Immediate hypersensitivity is mediated by IgE, which induces the degranulation of cells. The release of vasoactive substances and certain enzymes thereby causes symptoms. Delayed hypersensitivity is usually mediated by IgE and IgG, but mast cells and eosinophils also participate. The release of various bioactive substances causes the symptoms of allergic shock, a hypersensitive response in skin, and gastrointestinal hypersensitivity.[10]

Some drugs act as carriers or haptens to combine with carriers or haptens in the body. They can form complete antigens, which can stimulate the body to produce antibody, so type-II hypersensitivity is induced. Type-II hypersensitivity is induced mainly by IgG, IgM, and complement, and leads to the symptoms of hemolysis and neutropenia. An antigen that has continuous access to the body can stimulate the generation of antibodies, and immune complexes (ICs) arise, causing tissue damage, so type-III hypersensitivity is present.

Table 3 Effect of the anaphylactoid reaction on Beagle dogs

Group	*n*	Judgment of the typical behavior (score)						Histamine increase (%)	Blood pressure (mm Hg)	Outcome
		-	+	++	+++	++++	Mean			
1.44% histamine phosphate	3	0	0	0	0	3	3.0±0.0[a]	134.1±155.7[a]	-97.0±44.5[a]	Strong positive
NS	3	3	0	0	0	0	0.0±0.0	3.8±24.5	23.8±10.0	Negative
Baicalin	3	3	0	0	0	0	0.0±0.0	44.4±80.1	2.0±10.5	Negative
Honeysuckle	3	3	0	0	0	0	0.0±0.0	-5.7±41.3	0.9±19.2	Negative
Radix Isatidis	3	2	1	0	0	0	0.5±0.7	111.1±148.6[b]	6.6±8.1	Suspected
Gardenia jasminoides	3	2	0	1	0	0	0.7±1.2	11.6±75.8	11.9±28.5	Negative
Hydrolysate	3	2	1	0	0	0	0.3±0.6	39.4±51.0	3.5±12.8	Negative
Cholic acid	3	3	0	0	0	0	0.0±0.0	27.2±26.4	4.7±8.8	Negative
Hyodeoxycholic acid	3	3	0	0	0	0	0.0±0.0	163.2±241.0[aa]	-4.6±25.7	Suspected
Qingkailing injection	3	1	0	2	0	0	1.3±1.2	102.4±73.1[a]	-10.0±15.5	Positive

Notes: NS: negative control group with normal saline; Compared with the NS group, [a]*P*<0.01, [b]*P*<0.05. Evaluation criteria of anaphylaxis: positive in behavior: >2; suspect: 1-2; Positive in immunosubstance: >50%; suspect: 30%-50%.

Table 4 Effect of the allergic reaction on Beagle dogs

Group	*n*	Judgment of the typical behavior						Increasing percentage (%)		
		-	+	++	+++	++++	Mean	Histamine	IgE	IgG
1% egg protein	3	0	0	0	0	3	4±0	217.0±99.9	118.5±195.1	84.4±23.1[a]
NS	3	3	0	0	0	0	0±0	-10.7±28.2	1 3.9±29.1	-19.8±4.0
Baicalin	3	3	0	0	0	0	0±0	178.9±191.6	57.8±116.7	11.1±19.3
Honeysuckle	3	0	1	2	0	0	1.7±0.6	54.7±140.9	196.0±262.6	23.5±32.4
Radix Isatidis	3	0	1	1	0	0	1.5±0.7	107.5±66.7	96.1±111.5	71.4±2.6[a]
Gardenia jasminoides	3	2	1	0	0	0	0.3±0.6	35.2±88.2	129.2±159.1	37.4±72.6[b]
Hydrolysate	3	3	1	2	0	0	1.7±0.6	196.8±44.7	99.3±33.2	56.8±23.4[b]
Cholic acid	3	3	0	3	0	0	2±0	48.0±26.8	68.0±76.8	37.1±30.4
Hyodeoxycholic acid	3	3	0	0	0	0	0±0	-51.9±19.8	103.4±74.7	-39.9±24.0
Qingkailing injection	3	0	0	3	0	0	2±0	60.6±122.7	96.2±89.6	52.7±37.6[b]

Group	*n*	Increasing percentage (%)			Blood pressure (mm Hg)	Decision outcome
		IgM	IL-4	ECP		
1% egg protein	3	261.0±286.0[a]	137.8±61.4[b]	159.5±119.0[a]	-115.3±18.8[a]	Strong positive
NS	3	-42.5±32.0	-25.6±21.8	-18.9±24.5	7.2±24.5	Negative
Baicalin	3	40.1±64.4	154.0±133.1[a]	133.8±118.1[b]	13.9±18.2	Negative
Honeysuckle	3	67.1±61.7	29.6±35.0	64.2±36.2	5.6±24.2	Positive
Radix Isatidis	3	81.8±27.3	108.7±93.1	90.7±17.4	-10.7±12.0	Positive
Gardenia jasminoides	3	19.0±61.7	22.2±68.6	11.7±71.3	-10.7±15.8	Suspicious
Hydrolysate	3	58.7±64.9	122.6±78.6[b]	67.8±57.6	-1.9±23.8	Positive
Cholic acid	3	74.3±84.4	28.4±31.7	68.0±44.8	13.0±26.3	Positive
Hyodeoxycholic acid	3	8.9±66.4	-2.5±56.9	-34.1±43.2	8.9±12.7	Negative
Qingkailing injection	3	224.8±145.0[a]	87.3±56.8	131.2±72.4[b]	2.5±19.0	Positive

Notes: NS: negative control group with normal saline; LgE: immunoglobulin; IL-4: interleukin; ECP: eosinophil cationic protein; Compared with the NS group, [a]*P*<0.01, [b]*P*<0.05. Evaluation criteria of anaphylaxis: positive in behavior: >2; suspect: 1-2; Positive in immunosubstance: >50%; suspect: 30%-50%.

Type-III hypersensitivity is induced mainly by IgG, IgM, and immune complexes, leading to the symptoms of serum sickness and allergic shock.[10]

When allergens enter the body, B cells distinguish antigens and become activated. They then carry out endocytosis and process antigens. They combine with major histocompatibility complex (MHC) II, which releases IL-4 after it is recognized by helper T cells, and IL-4 further promotes B-cell activation. Activated B cells produce the corresponding specific IgE antibody, and the latter crosslinks with mast cells and eosinophils. Inflammatory mediators such as histamine, ECP, eosinophil peroxidase (EPO), and eosinophil neurotoxin (EDN) are released under the action of allergens, thereby resulting in symptoms.

The present study showed that the anaphylactoid reaction of Beagles was typical and the allergic reaction was not typical (particularly with regard to immune indices). In the allergy experiment, behavioral abnormalities were regarded as the main indices to judge the reaction, and the changes in the levels of histamine, IgE, IgG, IgM, ECP, and IL-4 were not in a regular pattern in the anaphylactoid reaction. In comparison, BN rats and guinea pigs are more suitable animals for experimentation in allergic reactions because they have evident behavior reactions and changes in immune indices.

When judging allergic reactions, in addition to noting immediate type-I hypersensitivity, the occurrence of delayed hypersensitivity as well as type-II and -III hypersensitivity should be noted. Hence, importance should be attached to histamine and immunological parameters such as IgE, IgG and IgM. ECP and IL-4 are important in signal transduction pathways and should be observed. The present study showed that QI and its components need to be refined further for ensuring safety in clinical applications.

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